are other SOx proteins responsible for S-S that remain undetected by our antibody. We need further studies to obtain the c-DNA clone of SOx to detect the other isoforms of the enzyme in the keratinocyte cDNA library.

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Abundant Expression of Vasoactive Intestinal Polypeptide Receptor $VPAC_2$ mRNA in Human Skin

To the Editor:

Vasoactive intestinal polypeptide (VIP) is a bioactive peptide that influences many aspects of cell function and differentiation. The 28-amino acid polypeptide belongs to the glucagon/secretin superfamily and is widely expressed in the central nervous system and in peripheral tissues including lung and skin, where it has been shown to have a multitude of biological functions (Dickinson and Fleetwood-Walker, 1999). VIP is abundantly present in cutaneous autonomic and sensory nerve fibers (Eedy *et al*, 1994) where it acts as neuromodulator and participates in the regulation of regional blood flow. As the expression and localization of $VPAC_2$ mRNA has not been examined so far, this study was carried out to correlate VIP and its receptor in the human skin. Surgically resected human skin samples (n = 24) were obtained from surplus remnants in excess of that required for pathologic examination after informed consent.

For mRNA *in situ* hybridization, $VPAC_2$ receptor cRNA probes were generated by a standardized protocol using the sequence corresponding to the region spanning from TM 3–7 of the human $VPAC_2$ receptor from TSUP1 human T lymphoblast cDNA which was subcloned into a specific vector (pGEM-T vector, Promega, Madison, WI) (Groneberg *et al*, 2001a). For antisense probes, plasmid linearization by restriction with Spe I was followed by transcription with T7 polymerase, for sense probes, the Nco I linearized plasmids were transcribed with SP6 polymerase (all Roche Diagnostics, Mannheim, Germany). The probes were checked for their integrity by TAE-agarose gel electrophoresis and ethidium bromide staining.

The $VPAC_2$ receptor mRNA distribution was assessed by nonisotopic *in situ* hybridization using a standardized protocol (Groneberg *et al*, 2001b) with 6–8 μm cryostat sections of 4% paraformaldehyde fixed biopsies.

High resolution interference contrast microscopy revealed abundant staining in all areas of the biopsies (**Fig 1**). Positive $VPAC_2$ mRNA-signals were localized to the cytoplasm of keratinocytes with a signal intensity that was maximal at the basal zone and decreasing to superficial layers (**Fig 1*A***). In the deep part of the dermis, $VPAC_2$ mRNA hybridization signals were present in cells of eccrine sweat glands and in cells of the germinative epithelium, matrix, and medulla of the hair follicle (**Fig 1*C***). Also, positive $VPAC_2$ mRNA hybridization signals were localized to endothelial and mononuclear immune cells. Control hybridizations on alternate sections with equivalent amounts of the digoxigenin labeled sense-probe using the same hybridization conditions and washing stringencies were unstained (**Fig 1*B*, *D***).

To relate the distribution of $VPAC_2$ mRNA to its ligand VIP, immunohistochemical studies were carried out using mouse polyclonal (1:1000, Biogenesis, Poole, U.K.) or monoclonal (1:100 Charles River, Southbridge, MA) VIP-antibodies as described before (Fischer *et al*, 1998) with 6–8 μm cryostat sections and antimouse fluorescein-5-isocyanate antiserum (1:400, Amersham, Braunschweig, Germany) and anti-rabbit biotin (1:200) – strepatavidin Texas Red (1:400, Amersham) as secondary antibodies.

Fluorescence microscopy demonstrated an abundant expression of VIP-immunoreactivity in nerve fibers which were found as branching networks surrounding blood vessels, eccrine sweat glands, and hair follicles (**Fig 1*E*** and ***F***) and in direct contact to $VPAC_2$ mRNA positive cells. Controls by omitting the primary or secondary antibodies and incubation with the preimmune serum did not reveal specific immunosignals.

VIP binding sites have so far been shown in sweat glands using unspecific binding techniques (Heinz-Erian *et al*, 1986) and in allergic contact dermatitis (Lundeberg and Nordlind, 1999). There is also a large body of evidence on the functional role of VIP as a possible sensory neuropeptide in normal skin and dermatologic disorders. VIP protein expression has been demonstrated in normal skin of young (Eedy *et al*, 1994) and elderly (Abdel-Rahman *et al*,

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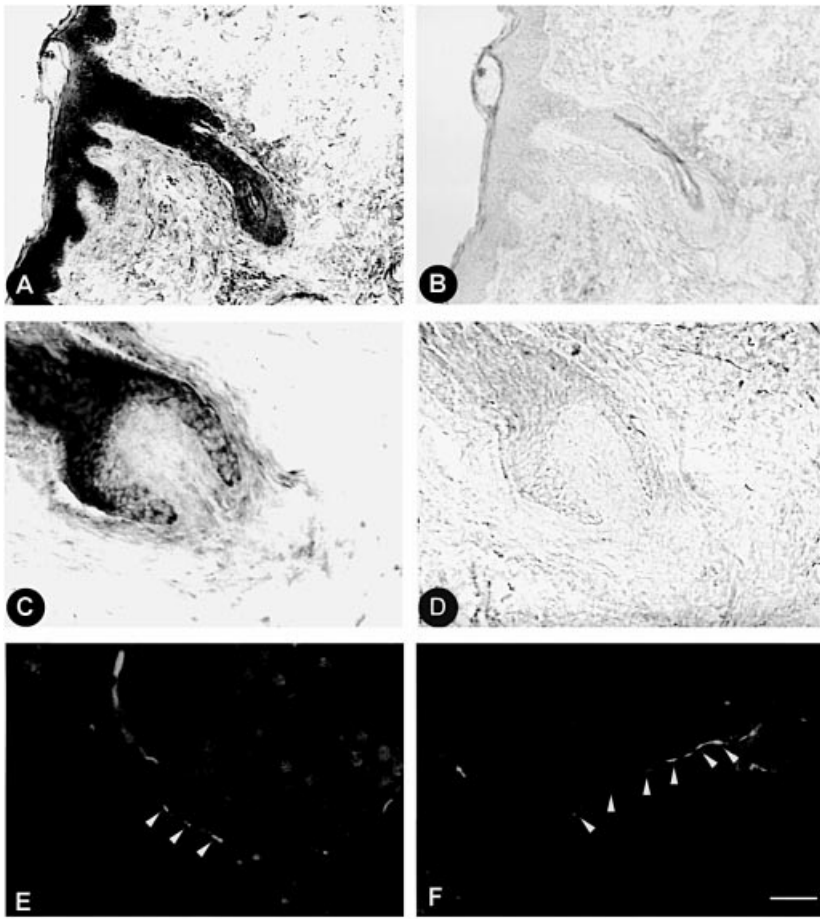

Figure 1. Localization of $VPAC_2$ receptor messenger RNA and VIP immunoreactivity. Eight micrometer cryostat sections of human skin were subjected to nonradioactive *in situ* hybridization for $VPAC_2$ mRNA. $VPAC_2$ mRNA hybridization signals are localized to epidermal keratinocytes (*A*) and cells of the germinative epithelium, matrix, and medulla of the hair follicle (*C*). Control slides, treated with the sense probe, did not show specific staining (*B*, *D*). Immunohistochemistry for VIP resulted in abundant staining of VIP-immunoreactive nerve fibers that were present around sweat glands (*E*) and in the subcutis (*F*). *Scale bars*: (*A*, *B*) 160 μm, (*C*, *D*) 60 μm, (*E*, *F*) 40 μm.

1992) people and in dermatologic disorders such as nodular prurigo (Abadia Molina *et al*, 1992) or allergic contact dermatitis (Lundeberg and Nordlind, 1999). Also the functional role of VIP in the normal skin and in disorders like contact dermatitis (Bondesson *et al*, 1996) was analyzed; however, a detailed study of the mRNA expression of molecular distinct VIP receptors has not been carried out.

As the molecular properties of the VIP receptors were identified in the past years, this study was designed to localize the mRNA of the inducible VIP receptor $VPAC_2$ in normal human skin biopsies and resulted in abundant staining for $VPAC_2$ mRNA in keratinocytes. Effects of VIP on keratinocyte cultures have been demonstrated earlier *in vitro* (Haegerstrand *et al*, 1989). This demonstration of $VPAC_2$ receptor mRNA in human keratinocytes *in situ* supports the previous *in vitro* findings and provides evidence that the mode of VIP modulation of cell proliferation in keratinocytes is mediated at least partly via the $VPAC_2$ receptor. This finding is also supported by the recent demonstration of $VPAC_2$ immunoreactivity in allergic contact dermatitis (Lundeberg and Nordlind, 1999).

A dense network of VIP-IR nerve fibers around sweat glands and hair follicles was shown in this study, and VIP has been reported to induce cAMP generation in human sweat glands (Tainio, 1987). By directly localizing $VPAC_2$ mRNA to eccrine gland and hair follicle cells we provide first evidence for the identity of the VIP receptor subtype that is involved in VIP-induced upregulation of eccrine sweat secretion.

VIP has been shown to be a potent relaxant of vascular smooth muscle (Lundberg *et al*, 1981). As $VPAC_2$ receptor mRNA was not present in the vascular smooth muscle layer of cutaneous vessels, our results indicate that VIP-induced dermal vasodilation is mediated via a different VIP receptor or by paracrine $VPAC_2$ stimulation. Also, the demonstration of $VPAC_2$ mRNA in mononuclear inflammatory cells is a novel finding with significant impact on the role of VIP in cutaneous immunomodulation, as a multitude of functional studies have suggested an important role of the peptide in the regulation of the immune system (Bellinger *et al*, 1996).

In conclusion, this study demonstrates an abundant expression of $VPAC_2$ mRNA in human skin that is associated to VIP immunoreactive nerve fibers and suggests a major participation of the $VPAC_2$ receptor in cutaneous VIP-signaling.

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